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ANALYSIS OF POLYETHERS BY ISOCRATIC HPLC WITH UNIVERSAL DETECTORS. III. A STUDY ON REPRODUCIBILITY

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ABSTRACT:

Liquid chromatography under critical conditions (LCCC) on ODS-columns in methanol-water separates ethoxylated fatty alcohols (FAE) and block copolymers of ethylene oxide (EO) and propylene oxide (PO) according to the length of the hydrophobic block. The elution behaviour of polyethylene glycols (PEG), polypropylene glycols (PPG) and fatty alcohols on different columns, in different mobile phase compositions, and at different temperatures is studied and the long-term reproducibility evaluated. It is shown, that - depending on the length of the blocks - the best separation need not be achieved exactly at the critical point of adsorption.

INTRODUCTION:

The analysis of non-ionic surfactants, such as ethoxylated fatty alcohols (FAE) and block copolymers of ethylene oxide (EO) and propylene oxide (PO), which are in widespread use for various applications [1], is complicated by the distributions of molar mass (MMD), chemical composition (CCD) and functionality (FTD).

A full characterization of these materials requires the determination of the distributions mentioned above. This can only be achieved by a combination of different chromatographic techniques, such as HPLC [2-19], GC [20-23], and SFC [11,24]. Recently, matrix-assisted laser desorption ionization time-of-flight mass spectroscopy (MALDI-TOF-MS) [25] has been combined with chromatography and yields valuable informations.

As has been pointed out in preceding papers [26-29], three different chromatographic modes can be applied to the analysis of nonionic surfactants:

Size Exclusion Chromatography (SEC) [30] separates according to molecular dimensions, provided, that adsorption effects can be excluded.

Normal phase HPLC [2,3,12,13,15,16] separates according to polar groups (EO-chain, OH-groups).

Reversed phase HPLC [7-11,17,18,26,29] separates according to hydrophobic groups (alkyl groups, PO-chain, in mobile phases with a high water content the EO-chain, too).

None of these techniques - if used alone - provides all required informations, even though multiple detection can enhance the analytical power of one-dimensional chromatography. The ultimate goal - the characterization of a sample in the form of a 3-dimensional map - can, however, only be reached by two-dimensional chromatography using two of the techniques mentioned above [31,32].

The most feasible approach involves a separation according to the non-polar part - the alkyl group or the poly(propylene oxide) chain - using liquid chromatography under critical conditions (LCCC) [31-34] for poly(ethylene oxide) (PEO) in the first dimension, and subsequent analysis of the pure homologous series thus obtained by SEC [28], normal phase HPLC [35], SFC or MALDI-TOF-MS [36].

In such a two-dimensional method, the problems with quantification can be solved, which arise from the dependence of response factors on molar mass and chemical composition, and from different preferential solvation of both blocks, as has been pointed out in previous communications [28,29,37-41].

In LCCC all members of a homologous series elute at the same volume, because entropic and enthalpic terms compensate each other (critical point of

adsorption). Hence the corresponding block (EO) becomes chromatographically invisible, and the separation occurs exclusively according to the length of the hydrophobic block.

In previous papers [26,27,29,39] we have shown, that this can be achieved on ODS columns in methanol-water at compositions between 80:20 and 90:10 (w/w).

As has been shown by several authors [42-49], the properties of alkyl bonded phases can be quite different, depending on the nature of the matrix (silica or polymer based) and on the surface density of the alkyl groups. Moreover, the properties of a column can change, when it is used for longer time in some mobile phases.

Hence we have studied the elution behaviour of polyethylene glycols (PEG), polypropylene glycols (PPG), fatty alcohols and FAE with respect to

1. the mobile phase composition corresponding to the critical point of adsorption for PEG
2. the reproducibility of the separation on different columns
3. the long-term reproducibility on the same column
4. the effect of operating temperature
5. the best composition of the mobile phase for different types of surfactants

In order to achieve a sufficient separation of homologous series with the same alkyl end group or the same number of PO-units, the difference of their elution volumes must be larger than the peak width of the PEG block. It should be shown, whether or not this is always the case exactly at the critical point of adsorption.

EXPERIMENTAL:

These investigations were performed using the density detection system DDS70 (commercially available from CHROMTECH, Graz, Austria), which has been developed in our group. This instrument has been described in full detail in previous communications [50-52]. The column box was thermostatted to 25.0°C and 30.0°C

using a Lauda MS-3 thermostat. The density detector cell was combined with a Bischoff 8110 RI detector and connected to a MS-DOS computer via the serial port. Data acquisition and processing was performed using the software package CHROMA [52], which has been developed for the DDS 70.

Several analytical and a semi-preparative column were used for these investigations.

In the following text the columns will be denoted by the following abbreviations:

- a) ODS2-10cm: Spherisorb ODS2 3 μm , 4.6*100 mm (PhaseSep)
- b) ODS2-prep: Spherisorb ODS2 5 μm , 10 *250 mm (PhaseSep)
- c) ODS2-3: Spherisorb ODS2 5 μm , 4.6*250 mm (PhaseSep)
- d) ODS2-4: Spherisorb ODS2 5 μm , 4.6*250 mm (PhaseSep)
- e) ODS B Spherisorb ODSB 5 μm , 4.6*250 mm (PhaseSep)
- f) Merck Lichrospher 100 RP 18, 5 μm , 4.6*100 mm (Merck)
- g) ACT-1 ACT-1, 10 μm , 4.6*150 mm (Interaction)

The flow rate was 0.5 ml/min on the 4.6 mm - columns and 2 ml/min on the 10 mm - column. Two JASCO 880 PU pumps were used: one for column a and one for columns b-g, which were connected to two Rheodyne 7060 column switching valves. Samples were injected using Rheodyne 7125 injection valves with a 50 and a 500 μl loop, respectively.

Fatty alcohols, polyethylene glycols, and polypropylene glycols were purchased from FLUKA or Aldrich and used without further purification. Methanol and water were HPLC grade (Merck, LiChroSolv). The mobile phases were mixed weight by weight and degassed in vacuo prior to use.

COMPARISON OF COLUMNS UNDER DIFFERENT CONDITIONS:

When looking for the critical point of adsorption for PEG, a comparison of different columns is rather easy: in this case it is sufficient to plot the elution volumes versus the molecular weight of the PEGs (or vice versa): under critical conditions the elution volumes should be constant.

In the analysis of PPG or fatty alcohols, a comparison of different columns requires a parameter, which is independent on column dimensions: the capacity factor k' of a substance, which is given by its elution volume V_e and the void volume V_0 :

$$k' = \frac{V_e - V_0}{V_0} \quad (1)$$

Within homologous series, $\ln k'$ increases linearly with the number n of repeating units, such as methylene groups, EO- or PO-units [44,53].

$$\ln k' = A + B.n \quad (2)$$

(A and B are constants for a given polymer in a given mobile phase)

In a binary eluents, the capacity factors depend on the (volume or weight) fraction of the organic component in the mobile phase

$$\ln k' = \ln k'_w + S.x \quad (3)$$

wherein k'_w is the (extrapolated) capacity factor in pure water, and S is the so-called solute acceleration factor [44,54].

On a given stationary phase, the capacity factor of a given substance depends on the change in free energy ΔG°_{sorp} , when the molecule is adsorbed and on the volume ratio ϕ of the stationary and the mobile phase]:

$$\ln k' = -\frac{\Delta G^\circ_{sorp}}{RT} + \ln \phi \quad (4)$$

With the corresponding changes in enthalpy ΔH°_{sorp} , and entropy ΔS°_{sorp} , one may write

$$\ln k' = -\frac{\Delta H^\circ_{sorp}}{RT} + \frac{\Delta S^\circ_{sorp}}{R} + \ln \phi \quad (5)$$

It is obvious, that capacity factors will depend on temperature. This may be especially important at the critical point of adsorption, where the enthalpic and the entropic terms compensate each other.

RESULTS AND DISCUSSION:

In the first step of these investigations we tried to find the critical point of adsorption for polyethylene glycols on different columns and studied the influence of temperature. Then we analyzed fatty alcohols and polypropylene glycols on the same columns and evaluated the reproducibility of the separation. Finally we tried to optimize the conditions for each separation problem.

Polyethylene glycols:

First of all we compared several octadecyl-columns in the analysis of PEGs in methanol-water 80:20 (w/w) at a temperature of 25.0°C. In Figure 1 the elution volumes thus obtained are plotted versus the molecular weight of the PEGs.

At the critical point of adsorption all PEGs should be eluted at the same volume, regardless which molecular weight they have. As can be seen, there are considerable differences between the columns, even between col.3 and 4, which come from the same producer, but from a different batch.

Figure 2 shows the results obtained on the same column (col.4), but in different mobile phase compositions: Obviously, the critical point of adsorption is reached on this column rather at a mobile phase composition above 90:10.

Figure 3 shows the results obtained on the 25 cm columns at two different mobile phase compositions.

In Figure 4 the corresponding results from the short columns (10 and 15 cm, respectively) are shown.

It is evident, that for three columns (ODS2-4, ODS2 10 cm, and Merck LiChroSpher ODS) the critical point of adsorption for PEG is reached at a composi-

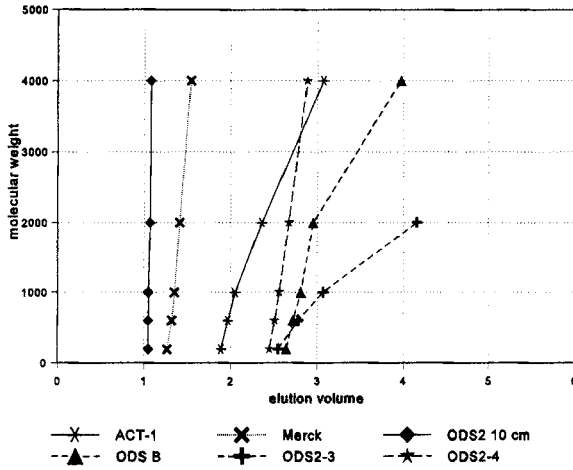


FIGURE 1:

Elution behaviour of polyethylene glycols on different columns in methanol-water 80:20 (w/w) at 25.0°C

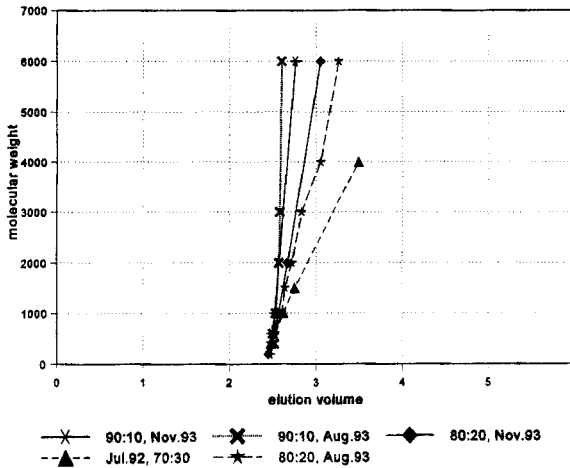


FIGURE 2:

Elution behaviour of polyethylene glycols on column ODS2-4 in methanol-water of different composition. Solid lines: new measurements, dashed lines: old measurements

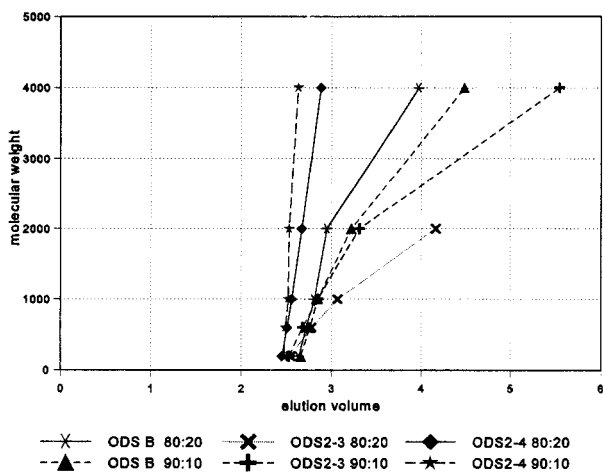


FIGURE 3:

Elution behaviour of polyethylene glycols on different columns in methanol-water of different composition

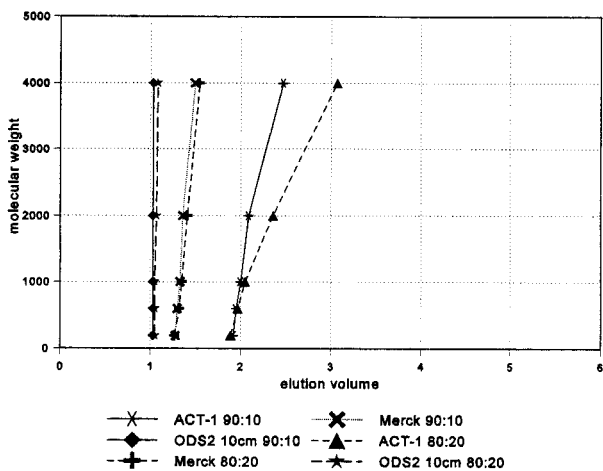


FIGURE 4:

Elution behaviour of polyethylene glycols on different columns in methanol-water of different composition

tion very close to 90:10, on ODS B somewhat higher. The difference between 80:20 and 90:10 is, however, not dramatic: at 80:20 the difference in elution volumes is still quite small (at least for lower molecular weights). The semi-preparative ODS2-column, which is not shown in these figures, is almost identical with ODS2-4 (most probably it comes from the same batch). For two columns (ODS2-3 and ACT-1) no critical point of adsorption for PEG is observed in methanol-water.

When the measurements in methanol-water 80:20 were performed at a temperature of 30.0°C, no considerable effect was observed for ODS2-4, as can be seen from Fig. 5. ODS B comes a little closer to the critical point of adsorption, while ODS2-3 is still far away.

Fatty alcohols:

Once the critical point of adsorption for PEG had been determined, we analyzed various fatty alcohols on different columns in methanol-water 90:10 (w/w). In Figure 6 the capacity factors thus obtained are plotted versus the carbon number of the alcohols. As can be seen, the differences between the columns are not very large.

In Figure 7, the results obtained on ODS2-10cm in methanol-water of different compositions are shown. The slopes of the lines can be used as a measure for the selectivity of the mobile phase. In a different representation of the same data, which is shown in Figure 8, it becomes clear, that no satisfactory separation is achieved in 100% methanol, while a composition of 90:10 will be suitable for higher alkanols ($n > 12$) and 85:15 or even 80:20 for lower alkanols.

Polypropylene glycols:

The next step was the analysis of polypropylene glycols up to molecular weights of 1200. The lower molecular samples (PPG 425, PPG 725) could not be analyzed in methanol-water 90:10, which would correspond to the critical point of adsorption for PEG, but in 80:20 a satisfactory separation was achieved.

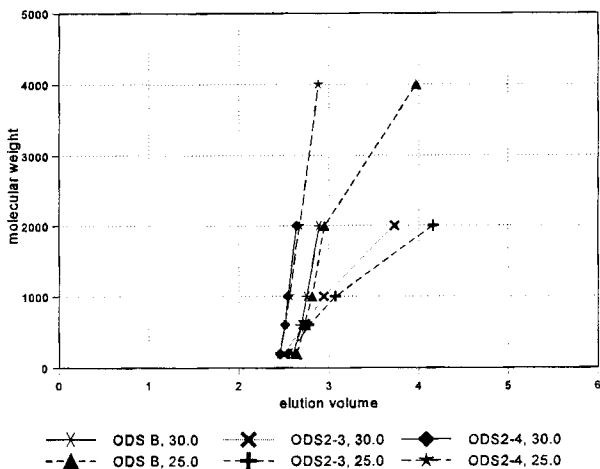


FIGURE 5:

Elution behaviour of polyethylene glycols in methanol-water 80:20(w/w) on different columns at different temperatures

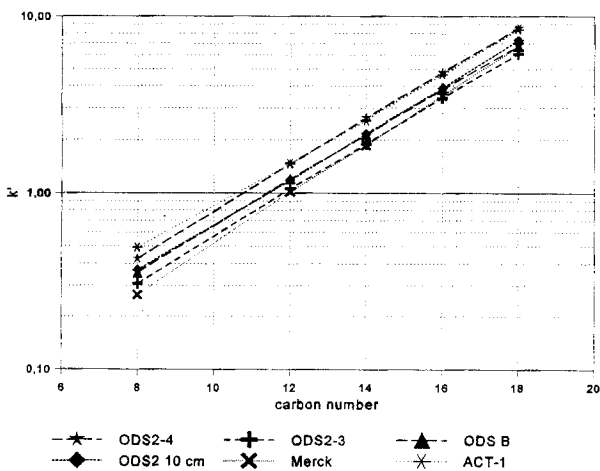


FIGURE 6:

Capacity factors of fatty alcohols on different columns in methanol-water 90:10 (w/w)

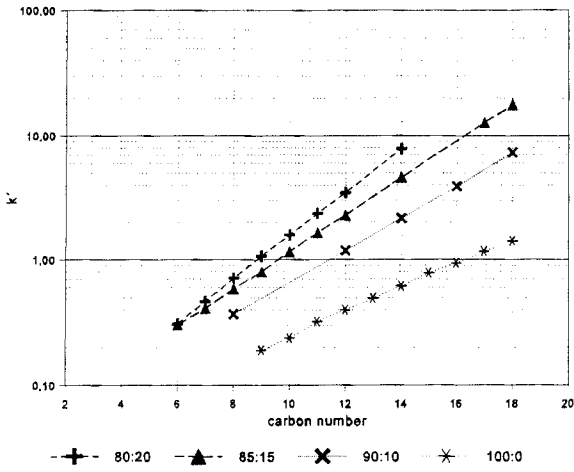


FIGURE 7:

Capacity factors of fatty alcohols on ODS2 10 cm in different mobile phase compositions

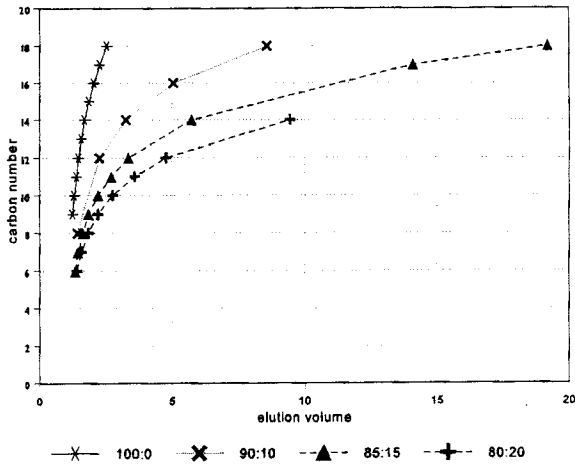


FIGURE 8:

Elution behaviour of fatty alcohols on ODS2 10 cm in methanol-water of different composition

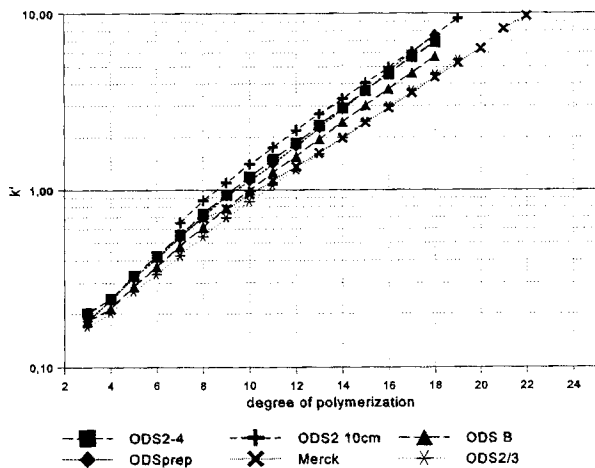


FIGURE 9:

Capacity factors of polypropylene glycols on different columns in methanol-water 80:20

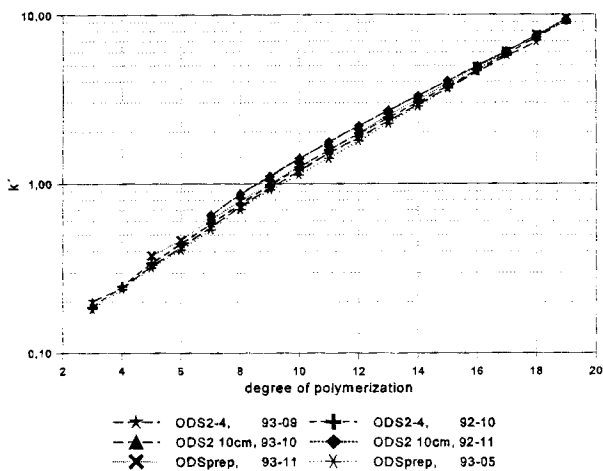


FIGURE 10:

Reproducibility of capacity factors of polypropylene glycols on three different columns in methanol-water 80:20 (w/w) over a period of 6 and 11 months, respectively

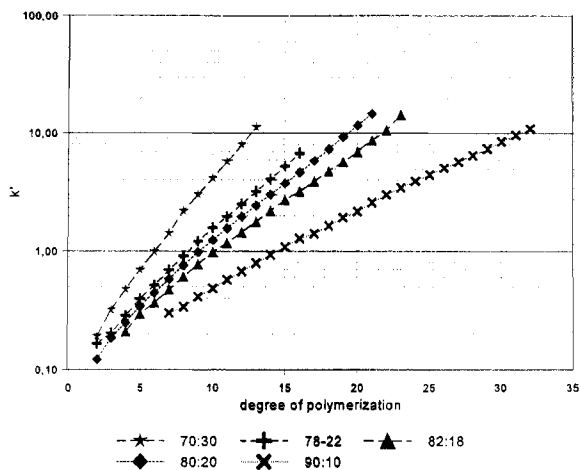


FIGURE 11:

Capacity factors of polypropylene glycols on ODS2-4 in different mobile phase compositions

In Figure 9, the capacity factors of polypropylene glycols on different columns in methanol-water 80:20 are plotted versus the degree of polymerization (the number of PO-units). Again the difference between the columns is not very large.

Figure 10 shows a comparison of data from three ODS2 columns of different dimensions. The data were obtained in the same mobile phase composition, but after a period of 6 or 11 months. As can be seen, not only the reproducibility between the columns is excellent, but also the long-term stability.

In Figure 11, the capacity factors of PPG on ODS2-4 in different mobile phase compositions are shown. The selectivity increases with decreasing water content, as expected.

In a different representation the elution behaviour of PPG is shown in Fig. 12: as already mentioned, 90:10 will only work well with PPG 1000 or 1200, for lower molecular weights 80:20 would be adequate. If the PEG-block of a copolymer is rather short, it might be reasonable to go even below this composition.

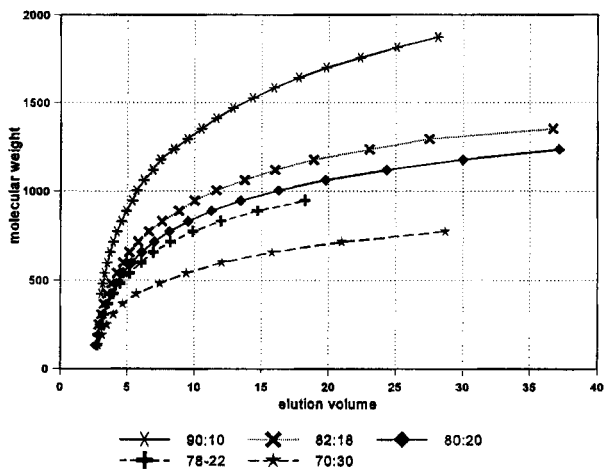


FIGURE 12:

Elution behaviour of polypropylene glycols on ODS2-4 in different mobile phase compositions

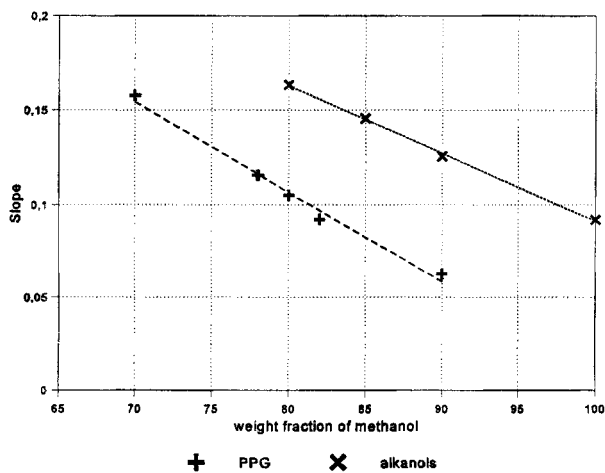


FIGURE 13:

Selectivity of methanol-water for polypropylene glycols and fatty alcohols on ODS2

Optimization of the mobile phase:

From the plots shown in figures 7 and 11, we have calculated the slopes B of the regression lines in equation 2, which represent the selectivity of a mobile phase for fatty alcohols and PPG.

In Figure 13, the slopes thus obtained have been plotted versus the composition of the mobile phase. From this diagram one can very easily determine the composition of the mobile phase yielding the required selectivity.

In general, the optimum will depend on the relative lengths of both blocks. If the PEG-block is rather short, only the hydrophobic block determine the mobile phase composition, if it is longer, the best composition must be closer to the critical point of adsorption, as will be shown in further communications.

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